Leading Edge
Review

Beyond the Sequence: Cellular Organization of Genome Function

Tom Misteli[1,*]
[1] National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA
*Correspondence: mistelit@mail.nih.gov
DOI 10.1016/j.cell.2007.01.028

Genomes are more than linear sequences. In vivo they exist as elaborate physical structures, and their functional properties are strongly determined by their cellular organization. I discuss here the functional relevance of spatial and temporal genome organization at three hierarchical levels: the organization of nuclear processes, the higher-order organization of the chromatin fiber, and the spatial arrangement of genomes within the cell nucleus. Recent insights into the cell biology of genomes have overturned long-held dogmas and have led to new models for many essential cellular processes, including gene expression and genome stability.

Introduction

We usually think of genomes abstractly as one-dimensional entities that are purely defined by their linear DNA sequences. Reality, of course, is far more complex. The DNA helix is folded hierarchically into several layers of higher-order structures that eventually form a chromosome (Woodcock, 2006). In this way, DNA is compacted and can be accommodated in the limiting space of the cell nucleus. The spatial arrangement of the chromatin fiber and the genome as a whole dramatically affects the function of DNA, and knowing the sequence of a genome is insufficient to understand its physiological function.

In addition to the complex arrangement of the genetic information itself, the cellular factors that read, copy, and maintain the genome are organized in sophisticated patterns within the cell nucleus (Lamond and Spector, 2003; Misteli, 2005). Many transcription factors, chromatin proteins, and RNA-processing factors are compartmentalized and accumulate in distinct nuclear domains; specific nuclear processes such as transcription and replication occur at spatially defined locations in the nucleus. The organizational properties of genomes and the machineries that act on them create an elaborate architectural environment in which genomes must function. How they do so is one of the great challenges in modern cell biology.

Uncovering the cell biology of genomes is fundamental. Although comparative genome analysis and large-scale mapping of genome features have yielded insights into the physiological role of genetic information, these efforts shed little light onto the Holy Grail of genome biology, namely the question of how genomes actually work in vivo. The elucidation of the cellular organization of genomes and its impact on genome regulation is a logical next step after the completion of sequencing projects. Understanding genome function within its architectural framework is also highly relevant for biotechnological applications that range from stem cell differentiation to somatic cloning and gene therapy as all of these processes involve massive reorganization of nuclear architecture. Knowledge of the functional interplay between genome organization and activity will significantly contribute to making these applications more efficient and controllable.

Cellular organization of genome function occurs at three hierarchical levels: the spatial and temporal organization of nuclear processes themselves, including transcription, RNA processing, DNA replication, and DNA repair; the organization of chromatin into higher-order domains; and the spatial arrangement of chromosomes and genes within the nuclear space. Each one of these levels has regulatory potential, and all are interdependent. Several simple questions serve as guideposts to unravel the complex structure-function interplay of the genome in the cell: How are genome processes and genomes organized in 3D space? What are the fundamental principles of organization? What are the molecular mechanisms that give rise to the organization patterns? What are the physiological consequences of spatial genome organization? Emerging answers to these questions are now leading to unprecedented insights into genome biology and to new, unexpected models of genome function.

Cellular Organization of Nuclear Processes

A hallmark of many nuclear processes is their spatial compartmentalization. Most nuclear events do not occur ubiquitously throughout the nucleus but are limited to specific, spatially defined sites that often occur in dedicated nuclear bodies (Lamond and Spector, 2003; Misteli, 2005). Remarkably, common mechanisms appear to organize some of the vastly different, fundamental nuclear processes.

The Organization of Transcription

The most fundamental of all genome functions is transcription. Surprisingly, there is still much uncertainty as to how transcription is organized within the nucleus (Cook, 1999; Chakalova et al., 2005). Visualization of

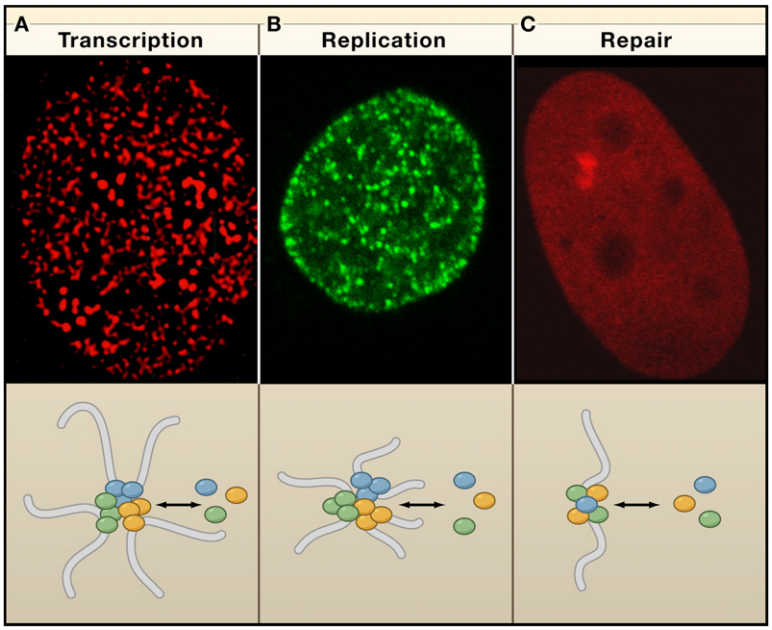

Figure 1. Compartmentalization of Nuclear Processes

Transcription, replication, and DNA repair are compartmentalized. (A) Transcription sites visualized by incorporation of bromo-UTP, (B) replication sites visualized by incorporation of bromo-dUTP, and (C) repair sites visualized by accumulation of repair factor 53BP1 at a double-strand break (DSB) are shown. In all cases, components are dynamically recruited from the nucleoplasm as single subunits or small preassembled subcomplexes. (A) is reprinted with permission from Elbi et al., 2002, (B) is courtesy of Rong Wu and David Gilbert at Florida State University, and (C) is courtesy of Evi Soutoglou from the National Cancer Institute, NIH.

transcription sites reveals the presence of several thousand distinct sites that appear to be randomly dispersed throughout the nuclear volume (Wansink et al., 1993; Figure 1A). Influenced largely by in vitro analysis of the transcription machinery, it was long assumed that this distribution represents RNA polymerases (RNA pol) elongating along genes. But an alternative and increasingly plausible view is that these sites correspond to subnuclear transcription centers (Cook, 1999; Chakalova et al., 2005; Figure 1A). As originally proposed by Cook, these "transcription factories" are transcription hot spots that harbor enough transcription factors and polymerases to serve multiple genes (Cook, 1999). The organization of transcription in centralized structures that contain multiple transcription machineries is consistent with the presence of an estimated 65,000 active RNA pol II molecules but fewer than 10,000 transcription sites in a HeLa cell. Considering that most active RNA pol II genes only contain one active polymerase at any time, transcription factories would contain between 6 and 8 actively elongating polymerases and would probably transcribe multiple genes at a time (Cook, 1999). The organization of RNA pol II transcription into distinct sites is not unprecedented, as it is analogous to the well-established clustering and compartmentalization of ribosomal RNA genes, which are transcribed by RNA pol I within the nucleolus in large, specialized transcription centers (Raska et al., 2006).

The compartmentalization of transcription has the obvious advantage of concentrating the required factors to ensure efficient interactions amongst components of the transcription machinery (Cook, 1999; Chakalova et al., 2005). An attractive possibility is that different transcription factories contain distinct sets of transcription components and thus create distinct transcriptional environments. Regulation of multiple genes may then be coordinated by their association with shared transcription factories of particular composition. At present, however, this idea is largely hypothetical as little evidence exists for differential composition amongst transcription sites.

The Dynamic Nature of Transcription Complexes

Despite the organization of transcription into structural compartments, biochemical-analysis and in vivo-imaging approaches have recently revealed that the transcription machinery is surprisingly dynamic and significantly determined by stochastic events (Misteli, 2005). These properties are based on the highly transient interaction of proteins with chromatin. In vivo analysis of many transcription factors and chromatin proteins suggests that most of them undergo rapid cycles of binding and unbinding on chromatin, with dwell times on the order of only a few seconds (Phair et al., 2004). Upon unbinding, proteins are able to freely diffuse through the nuclear space, which allows them to scan the genome for specific binding sites by using a hit-and-run mechanism (Misteli, 2001b; Hager et al., 2002).

Direct evidence for the highly dynamic nature of transcription complexes comes from observing transcription factors on their specific target genes in living cells. The glucocorticoid- and estrogen-receptor transcriptional coactivators bind to their specific response elements in the promoter regions of target genes with residence times on the order of only a few seconds (McNally et al., 2000; Stenoien et al., 2001), and several of their interaction partners bind equally transiently to the promoter (Becker et al., 2002). In addition, binding of NFκB on its cognate sites is highly transient (Bosisio et al., 2006). These findings on artificial promoter arrays are strongly corroborated by analysis of the assembly dynamics of RNA pol I subunits on endogenous ribosomal RNA genes (Dundr et al., 2002).

All RNA pol I subunits undergo rapid exchange at the promoter and stably associate with chromatin only when they are incorporated into an elongation complex. Assembly of the polymerase appears to occur in a stepwise process by largely stochastic collisions of subunits with the polymerase machinery at the promoter (Dundr et al., 2002). Further evidence for dynamic subunit assembly of transcription complexes comes from observation in *Drosophila*, where heat shock factor (HSF) becomes rapidly recruited and immobilized on its target genes upon heat shock, and HSF dynamics appear to differ from the polymerase proper (Yao et al., 2006). These observations challenge the traditional view of the holoenzyme being recruited to a gene in a single step, although they do not rule out that assembly occurs, at least in part, from preassembled subcomplexes (Schneider and Nomura, 2004).

Organization of DNA-Replication and -Repair Sites

The organization of transcription into distinct sites and their highly dynamic nature might be surprising at first, particularly in light of the more static view from traditional in vitro experiments. But similar principles of organization and dynamics also apply to other essential nuclear processes including DNA replication and repair.

Replication occurs at nuclear sites referred to as ''replication factories'' (Cook, 2002; Figure 1B). These factories associate with multiple replication origins and contain the entire replication machinery as well as additional factors involved in chromatin assembly and cell-cycle regulation. In a manner similar to transcription factories, replication factories form by recruitment of replication factors from an unbound, freely diffusing nucleoplasmic pool during S phase, and assembly occurs in a stochastic fashion from single subunits rather than from recruitment of preassembled replication machineries (Sporbert et al., 2002; Figure 1B). Once assembled, some components of the replication machinery, including the PCNA clamp, are stably incorporated for the duration of the replication cycle, which is typically on the order of a few minutes, whereas others rapidly exchange with the nucleoplasm (Sporbert et al., 2002; McNairn et al., 2005). The differences in residence times most likely reflect the specific temporal requirement of each factor in the replication process. The formation and maintenance of replication factories is entirely driven by the replication process alone, which strongly suggests that the replication factories are self-organizing structures (Kitamura et al., 2006).

The highly dynamic nature of replication factories is critical for their proper function as the plasticity of these sites is essential for progression of replication along chromosomes. Elegant photobleaching studies have demonstrated that a replication factory persists for a few minutes before it disassembles (Sporbert et al., 2002). A new factory is then assembled de novo from the unbound pool of factors. Remarkably, the new factory forms immediately adjacent to the previous one, thus ensuring ordered spreading of replication. The ability of the replication machinery to progress thus relies entirely on the dynamic nature of replication foci and their ability to rapidly disassemble and then reassemble at a new site (Sporbert et al., 2002).

DNA repair is similarly compartmentalized. It involves the rapid recruitment to sites of damage of key factors from a diffuse pool to form spatially defined repair foci in which DNA repair eventually occurs (Essers et al., 2006; Figure 1C). These repair centers may form at a single site of DNA damage, but observations in *Saccharomyces cerevisiae* suggest that a repair focus may also serve multiple damaged sites (Lisby et al., 2003). Whether this also applies to mammalian cells remains to be seen. Repair foci form in a highly dynamic fashion, and factors rapidly accumulate at damage sites upon induction of double-strand breaks (DSBs; Houtsmuller et al., 1999; Politi et al., 2005; Bekker-Jensen et al., 2006). The recruitment of a multitude of factors occurs from single subunits rather than from preassembled repair machinery as demonstrated by the differential kinetics of recruitment of various factors (Politi et al., 2005). As for replication and transcription factors, recruitment is not a directed process but occurs via the capturing of freely diffusing molecules from the nucleoplasm. The repair factors remain associated with the repair centers for various periods of time depending on their function and then diffuse away once they have completed their task (Houtsmuller et al., 1999; Politi et al., 2005).

The Stochastic, Self-Organizing Nature of Nuclear Processes

The emerging view from these studies is that assembly of large macromolecular complexes on chromatin occurs via recruitment of soluble subunits from a nucleoplasmic pool. This is accomplished by stochastic interactions of single subunits or small preformed subcomplexes (Misteli, 2001b; Figure 1). Stochastic assembly from subunits intuitively seems to be an inefficient way to ensure the establishment of functional machinery. However, given the ability of most nuclear proteins to rapidly roam the nucleus for specific binding sites, even relatively low-abundance proteins frequently encounter specific target sites (Misteli, 2001b). Considering that many genes only fire sporadically and that most replication and repair sites only require the presence of a few copies of a particular component, probabilistic interactions of factors with chromatin are sufficient to sustain their functionality. In addition, although the binding of each single subunit may be relatively inefficient, the presence of assembled intermediates most likely facilitates the incorporation of subsequent subunits into an assembling complex in a cooperative fashion (Dundr et al., 2002; Agresti et al., 2005).

An important and often neglected factor that facilitates stochastic interactions and makes them more efficient in vivo is molecular crowding. The estimated protein concentration in the nucleus is an exceedingly high 100–400 mg/ml. In addition, within the nonhomogenous topology of chromatin and nuclear bodies, molecules may be spatially trapped and corralled, which further favors their stochastic interactions. Molecular crowding greatly increases the effective concentration of a component by

several orders of magnitude, and rates of protein-protein and protein-DNA interactions are greatly elevated (Minton, 2000).

An additional property of molecularly crowded systems is the emergence of discrete phases that are formed by dynamic protein aggregates. Theoretical considerations indeed suggest that molecular crowding may be the driving force behind the formation of transcription and replication factories (Minton, 2000; Marenduzzo et al., 2006). Experimental evidence for a significant role of molecular crowding in the nucleus comes from the observation that expansion of the nuclear volume leads to the disassembly of several nuclear compartments, such as the nucleolus, as well as inhibition of nuclear processes including RNA pol I transcription (Hancock, 2004). Remarkably, introduction of inert macromolecules restores these structures morphologically and rescues RNA pol I transcription (Hancock, 2004). The absence of molecular crowding is likely one of the key factors for the dramatically reduced efficiency of in vitro transcription, splicing, and replication systems compared to that of the in vivo situation. The precise role of molecular crowding in gene expression remains to be elucidated.

Several organizational properties of transcription, replication, and repair strongly point to the possibility that the compartmentalization of these essential nuclear processes occurs via self-organization (Misteli, 2001a). All processes occur in highly dynamic steady-state structures, and the formation of the functional compartments is entirely dependent on their respective functions. Replication factories do not exist outside of S phase and form rapidly as cells initiate replication (Sporbert et al., 2002; Kitamura et al., 2006). In addition, their formation kinetics correlate with the rate of replication progression (Kitamura et al., 2006). Similarly, repair foci form rapidly upon induction of DNA damage, and their extent is related to the degree of global damage (Bekker-Jensen et al., 2006). Although the situation is less clear for transcription, we know that RNA pol I-mediated expression of ribosomal RNA gene clusters is sufficient to give rise to the nucleolus, which is one of the most prominent nuclear compartments (Karpen et al., 1988; Misteli, 2001a). These properties are hallmarks of self-organizing structures.

The similarities in spatial and temporal properties of the various nuclear processes indicate that the organizational principles involved in their biogenesis are universal. In fact, it seems likely that the same principles apply to virtually all nuclear structures as many nuclear bodies, including the nucleolus, Cajal bodies, PML bodies, and splicing-factor speckles, all share a high degree of dynamic protein exchange and stochastically recruit factors from the nucleoplasm, which is reminiscent of the dynamic behavior of transcription, replication, and repair sites (Misteli, 2005). It thus appears that compartmentalization of nuclear processes, likely via self-organization, into well-defined yet dynamically malleable sites is one of the fundamental principles of organizing genome function in vivo.

Higher-Order Chromatin Organization

Chromatin is organized into higher-order structures, although much of the details of the folding geometry are unclear (Cremer et al., 2006; Woodcock, 2006). It is known that the 10 nm nucleosomal fiber is folded helically into a fiber of around 30 nm and further into a 60–130 nm chromonema fiber. The characteristics of the fiber beyond this level have not been resolved. An indication for subsequent organization levels comes from the observation that early- and late-replicating chromosome domains of about 1 Mb in size are physically separate and are maintained over several cell cycles (Sadoni et al., 1999; Cremer et al., 2006). In addition, gene-rich and gene-poor stretches of chromosomes are physically separated from each other (Boutanaev et al., 2005; Shopland et al., 2006). Regardless of the precise geometry of higher-order chromatin, the folding of the fiber is critically important for genome function.

Chromatin as an Accessibility Barrier

A link between gene activity and chromatin structure originates from the observation that active genes are often found in largely decondensed euchromatin and silenced genes in condensed heterochromatin. The most common view for how chromatin folding may act as a regulatory mechanism is via preventing the access of regulatory factors by excluding them from condensed chromatin domains (Dillon and Festenstein, 2002). Although this model is attractive it is probably an oversimplification. Several large-scale mapping studies have found an incomplete correlation between gene activity and higher-order chromatin condensation. Comparison of gene-expression profiles with chromatin structure after biochemical separation of open and condensed regions reveals a correlation with gene density, rather than activity, with decondensed chromatin representing gene-rich regions and condensed regions gene-poor stretches of the genome (Gilbert et al., 2004). Similarly, higher-order chromatin condensation and gene expression only weakly correlate when probed by genome-wide micrococcal nuclease and DNase mapping (Sabo et al., 2004; Weil et al., 2004).

The idea of higher-order structure as a regulator of accessibility is also challenged by recent observations of the diffusional mobilities of proteins in the nucleus. In both *S. cerevisiae* and mammalian cells, heterochromatin proteins can readily diffuse into and bind to their sites in highly condensed heterochromatin (Cheutin et al., 2003, 2004; Festenstein et al., 2003). Similarly, inert diffusion probes that correspond to macromolecular complexes of several hundred kilodaltons can gain ready access to condensed chromatin (Verschure et al., 2003; Gorisch et al., 2005). These observations strongly suggest that the higher-order folding of chromatin per se does not present an insurmountable accessibility barrier to nuclear proteins and that the true accessibility barrier in chromatin lies at the level of the 10 nm nucleosome fiber or below.

Genome Regulation via Local Chromatin Loops

Chromatin loops are a ubiquitous structural element of chromatin (van Driel et al., 2003; Fraser, 2006; Figure 2).

They are attractive organizational and regulatory features because they provide structural support to the chromatin fiber and at the same time bring distantly located sequence elements into spatial proximity, which allows for regulatory communication between these sites. Vice versa, loops can spatially segregate genome regions from each other and ensure their independent function. Loops have been implicated in virtually all levels of chromatin organization and function ranging from kilobase-sized loops involved in the interaction of upstream elements with promoters to giant loops of hundreds of kilobases that might contribute to gene placement away from the chromosome body and into distinct nuclear environments (van Driel et al., 2003; Cremer et al., 2006). The existence and physiological relevance of the various types of loops is at times difficult to ascertain as they often cannot be detected under native conditions and are generally refractory to visualization in situ. Regardless, the relevance of loops in several gene-regulatory events has recently been reinforced (Fraser, 2006).

Local chromatin loops are critical in both positive and negative gene regulation (Fraser, 2006; Figure 2A). The prototypical example is the β-globin gene, whose enhancer physically interacts with the main body of the gene ~50 kb downstream concomitantly with activation (Wijgerde et al., 1995). Loop formation is not merely a consequence of transcriptional activation given that it occurs prior to gene activation when erythroid progenitor cells become lineage committed (Palstra et al., 2003). The purpose of looping is to bring together far-upstream locus-control regions, promoter-proximal regulatory elements, and the gene body itself to form a "transcription hub" that presumably creates an environment of high transcriptional activity by concentrating relevant transcription factors. In an extension of this idea, the thymocyte-specific SATB1 protein is responsible for tethering regulatory sequences of a number of target genes via formation of a multitude of loops, whose formation is directly linked to the proper regulation of the target genes (Cai et al., 2003, 2006). Chromatin loops may also contribute to gene silencing, as looping of imprinting-specific regions occurs parent specifically in the insulin-like growth factor 2, H19 gene cluster (Murrell et al., 2004), and the maternally expressed *DLX5* locus (Horike et al., 2005).

Chromatin looping might in fact be more prevalent and important for proper gene expression than is commonly thought. Recent analysis of the in vivo topology of several genes in *S. cerevisiae* and in humans suggests that active loci fold back onto themselves, bringing their 3′ end in physical proximity to their 5′ beginning (O'Sullivan et al., 2004; Ansari and Hampsey, 2005; Martin et al., 2005; Figure 2B). This behavior is consistent with the now widely accepted view that 3′ end-processing and RNA-processing factors physically interact with the transcription machinery (Bentley, 2005). Furthermore, gene looping explains the observation that termination- and 3′ end-processing factors often affect transcription and have been found to interact with promoter regions. Gene looping would provide an effective way to coordinate transcription and RNA processing and would facilitate reinitiation. The possibility that local looping is a general characteristic of active genes is of particular interest in light of the transcription-factory model in which the transcription machinery is concentrated in distinct sites from which genes loop out (Fraser, 2006). How prevalent looping of active genes is remains to be seen.

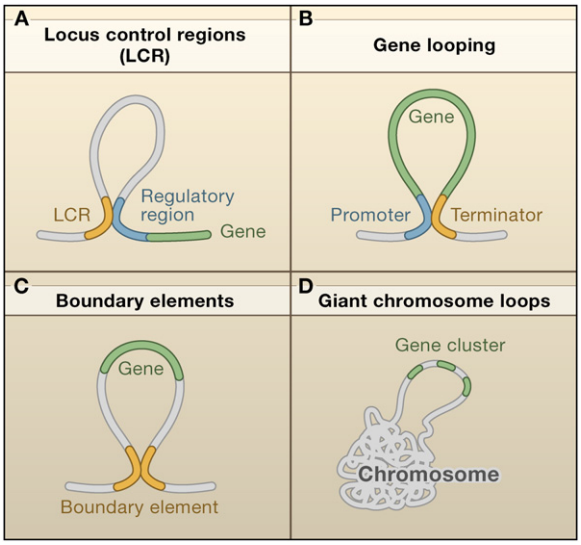

Figure 2. Local Organization of Chromatin
Local chromatin loops are essential for (A) transcriptional activation and repression, (B) coordination of initiation and termination/3′ end processing, and (C) boundary function. (D) Giant loops displace gene clusters from the chromosome body.

Local chromatin looping is likely also involved in maintaining the individuality and specific gene-expression properties of neighboring genes and genome regions (Labrador and Corces, 2002). Insulators and boundary elements are operationally defined gene-flanking sequences, and they protect a locus from the influence of its neighbors. One model to do so envisions the physical interaction of the insulator sequences generating a loop that contains the gene (Figure 2C). Physical interaction between flanking insulator regions has been demonstrated for the *Drosophila* scs and scs′ insulators (Blanton et al., 2003). Furthermore, the looping out of sequences located between two gypsy insulators can be visualized by light microscopy, and the introduction of an extra gypsy insulator into the loop leads to the formation of two smaller loops, which strongly suggests that the gypsy insulators form the basis of the loops (Byrd and Corces, 2003). Loops may also define the boundary between heterochromatic and euchromatic regions of the genome. In *Schizosaccharomyces pombe*, such boundaries are frequently characterized by binding of the RNA pol III transcription initiation factor TFIIIC, which localizes in several foci at the nuclear periphery. It has been suggested that boundary regions are clustered in TFIIIC foci, thereby organizing the

intervening, active sequences into loops that protrude into the nuclear interior (Noma et al., 2006).

The Emergence of Large-Scale Chromatin Loops

In addition to local loops, larger chromatin loops are emerging as possible candidates to contribute to genome regulation (Chubb and Bickmore, 2003; Cremer et al., 2006; Figure 2D). Giant loops of several megabases that emanate from the chromosome body have been suggested to represent a fundamental organization unit of chromatin (Chubb and Bickmore, 2003; Cremer et al., 2006). These loops are thought to segregate genome regions from each other and place them in distinct nuclear environments, presumably to optimize their activity. The most prominent examples of giant loops are highly expressed gene clusters such as the human major histocompatibility complex II and the mouse epidermal differentiation complex (Volpi et al., 2000; Williams et al., 2002). Both of these regions become expelled from their chromosome territory upon activation. Similarly, extrachromosomal loops are induced upon activation of the mouse Hox cluster (Chambeyron and Bickmore, 2004). The remarkable synchrony of this movement with the activation kinetics of the Hox cluster strongly suggests a functional link, although the precise role of this dramatic change is unknown (Chambeyron and Bickmore, 2004). Even though it is clear that neither all highly transcribed regions nor all transcribed gene clusters form giant loops, a recent high-resolution in situ hybridization method revealed a higher degree of intermingling between neighboring chromosomes than previously was assumed, which suggests that large chromatin loops might be more prevalent than commonly anticipated (Branco and Pombo, 2006). The recent development of methods to probe the physical association of genome regions in a unbiased and genome-wide scale should lead to rapid progress in our still-rudimentary understanding of the functional significance of chromatin loops (Simonis et al., 2006; Wurtele and Chartrand, 2006; Zhao et al., 2006).

A defining feature of all chromatin loops is their requirement for a tether at their base. Tethering occurs by several mechanisms. The gypsy-insulator and TFIIIC bodies are generally found associated with the nuclear periphery, which allows for the possibility of tethering to the nuclear edge (Byrd and Corces, 2003; Noma et al., 2006). The nuclear pore may serve as a tether given that synthetic boundary constructs interact with the nuclear-pore complex in *S. cerevisiae* (Ishii et al., 2002). On the other hand, an array of the chicken HS4 insulator and its flanking sequences associates with the nucleolus, and this localization is mediated by CTCF, one of the major insulator-binding proteins (Yusufzai et al., 2004). An intriguing possibility is that transcription and replication factories themselves may serve as bases of loops (Cook, 2002; Chakalova et al., 2005). Transcription and replication sites may in fact be the major tethering sources for chromatin loops as they are highly abundant and found throughout the nucleus. Furthermore, theoretical analysis of the entropy involved in the formation of loops by tethering to DNA and RNA pol clusters suggests that these are energetically favorable arrangements (Marenduzzo et al., 2006). Evidence for polymerase-mediated loops exists in both prokaryotes and eukaryotes ranging from yeast to *Drosophila* to humans (Cook, 2002). This indicates that transcription site- and replication site-mediated loop formation may be a universal and intrinsic principle of chromatin organization in the cell nucleus.

Spatial Organization of Genomes

The most global level of cellular genome organization is the arrangement of genome regions within the 3D space of the cell nucleus (Cremer et al., 2006; Meaburn and Misteli, 2007). The nonrandom nature of spatial genome organization is indicated by the age-old observation of segregation of transcriptionally active and inactive regions into physically separate domains of euchromatin and heterochromatin, respectively. Recent more-detailed mapping studies of smaller genome regions have significantly extended this concept and have made it clear that chromosomes, genome regions, and single genes are nonrandomly arranged within the nucleus (Cremer et al., 2006). Changes in positioning patterns occur during differentiation and development, which strongly suggests a link between positioning and genome function (Parada et al., 2004; Cremer et al., 2006).

Internal versus Peripheral Genome Positioning

A simple way to assess the position of a genome region within the nucleus is by determining its distance from the nuclear periphery. A general correlation between transcriptional silencing and localization toward the nuclear edge has long been suggested based on the observation that early-replicating and presumably transcriptionally active R bands are generally found toward the center of the nucleus, whereas late-replicating, inactive G bands are often located toward the periphery (Ferreira et al., 1997; Sadoni et al., 1999; Gilbert et al., 2004). In addition, human lymphocytes show a strong correlation between the radial position of human chromosomes and their gene density, with gene-poor chromosomes positioned toward the nuclear periphery and gene-rich chromosomes located in the nuclear interior (Boyle et al., 2001). Although similar correlations have been made in other cell types, chromosome positioning has been correlated with properties other than gene density, such as chromosome size (Bolzer et al., 2005). However, gene density or chromosome size alone clearly cannot explain the position of a chromosome given that the position differs between cell types and tissues where these properties are unchanged (Cremer et al., 2003; Parada et al., 2004).

Similar to that of chromosomes, the position of single genes relative to the nuclear periphery is nonrandom and has been linked to their functional status. For example, the IgH locus is preferentially associated with the nuclear periphery in B cell progenitors where it is silent, but it moves toward the interior when it becomes potentiated in B cell precursors (Kosak et al., 2002; Ragoczy et al., 2006). Similarly, the CD4 locus repositions from the periphery to

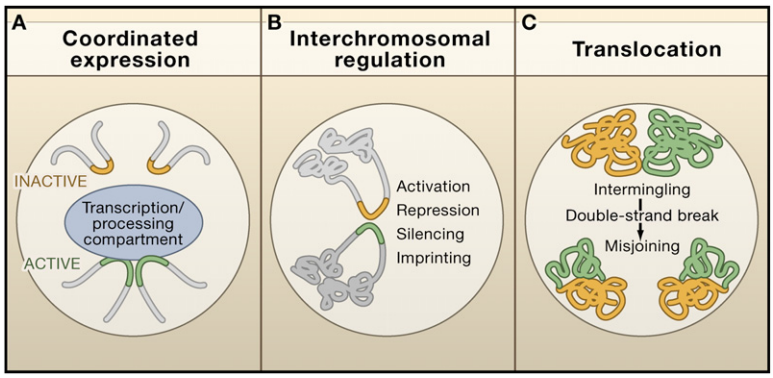

Figure 3. Functional Consequences of Global Chromatin Organization

(A and B) Spatial clustering of genes on distinct chromosomes facilitates their expression by (A) association with shared transcription and processing sites or (B) physical interactions with regulatory elements on separate chromosomes. (C) The physical proximity of chromosomes contributes to the probability of chromosomal translocations.

the nuclear interior during T cell differentiation, and Hox1b and Hox9 become internalized roughly concomitantly with their transcriptional activation (Chambeyron and Bickmore, 2004; Kim et al., 2004). On the other hand, the radial position of a gene is generally not directly related to its activity as indicated by the fact that in most cells the two alleles are positioned differently yet their functional properties appear to be similar (Roix et al., 2003). In addition, in many cases, no repositioning occurs upon a change in gene activity.

An extreme case of positioning is the physical association of gene loci with the nuclear periphery. In *S. cerevisiae*, association with the periphery is sufficient, although not necessary, for transcriptional silencing and increases DNA-repair efficiency (Gartenberg et al., 2004; Therizols et al., 2006). In mammalian cells, transcriptional activity of the cystic fibrosis disease gene correlates strongly with its association with the nuclear envelope (Zink et al., 2004). The nuclear periphery, however, does not function exclusively as a repressive environment given that a large number of *S. cerevisiae* genes are repositioned to the periphery where they interact with nuclear-pore components when they become activated (Brickner and Walter, 2004; Casolari et al., 2004; Cabal et al., 2006). This association with the periphery does not appear to be absolutely essential for their expression, but it might primarily play a role in optimizing gene activity (Taddei et al., 2006).

The potential role of the nuclear periphery in genome regulation has become of particular importance due to the emergence of several human diseases that are caused by mutations in the *LMNA* gene, which encodes lamin A and lamin C, the two major architectural proteins of the peripheral lamina (Gruenbaum et al., 2005). Although the nuclear lamina has traditionally been considered to have purely structural properties, recent observations allow for the possibility that it more directly contributes to gene regulation by tethering specific genome regions. In *Drosophila*, defined genome regions containing clusters of closely spaced genes have been identified that preferentially associate with the periphery and whose expression is affected by this interaction (Pickersgill et al., 2006). Peripheral localization of genome regions might occur directly via interactions between lamin A and core histones or more indirectly via chromatin-adaptor proteins (Gruenbaum et al., 2005). Interestingly, a hallmark of at least one of the lamin A-mediated genetic diseases is the dramatic change in histone-modification patterns and the almost complete loss of heterochromatin (Scaffidi and Misteli, 2005; Shumaker et al., 2006). How the nuclear lamina affects chromatin structure and epigenetic status is one of the most intriguing questions in the field.

Relative Positioning: The Power of Proximity

In contrast to the somewhat uncertain role of radial positioning, the position of multiple genome elements relative to each other is rapidly emerging as an important determinant of function (Figure 3).

For a long time, the lone example of spatial gene clustering had been the ribosomal genes, which coalesce in the nucleolus to bring the ribosomal gene arrays located on several separate chromosomes into physical proximity. More recently, similar coalescence has been described for tRNA genes in *S. cerevisiae* (Thompson et al., 2003). Initial evidence for spatial clustering of RNA pol II-transcribed genes in mammalian cells has recently come from the observation of colocalization of coordinately activated genes in erythroid cells (Osborne et al., 2004). Upon transcriptional activation, multiple genes that were located over 30 Mb apart on the same chromosome relocalized and became associated with shared transcription sites. Similarly, the human α- and β-globin genes located on chromosomes 16 and 11, respectively, are in close spatial proximity when highly expressed, thus extending the concept of gene clustering to multiple chromosomes (Brown et al., 2006; Figure 3A).

Although the functional significance of association of multiple coregulated gene loci is still unclear, direct physical interactions between chromosomes are now known to have regulatory functions (Figure 3B). This new paradigm was recently established by analysis of the *Ifng* and *TH2* loci in naive T cells (Spilianakis et al., 2005). The TH2 locus control region on mouse chromosome 11 physically interacts with the Ifng locus on chromosome 10 in naive T-helper cells. Upon stimulation of naive T cells to differentiate, the two genome regions separate, and *Ifng* transcription commences. Similarly, in sensory neurons a single odorant receptor from a large repertoire is selected

for expression by physical association of an odorant-receptor-enhancer element on chromosome 14, with the selected receptor localized on another chromosome (Lomvardas et al., 2006; Figure 3B). These observations establish the concept of *trans*-regulation via interchromosomal communication and suggest that, in addition to the physical interactions amongst genome elements on the same chromatin fiber, interactions in *trans* between regulatory elements on separate chromosomes must be considered in transcriptional regulation. A slight complication with these observations is the fact that associations are generally only observed for single alleles and not in all cells of a population. It is possible that this is a reflection of the dynamic nature of gene loci, which are able to move over several micrometers by constrained diffusion (Chubb and Bickmore, 2003). Alternatively, differences between alleles may be due to the stochastic nature of gene expression in which one allele is not transcribed continuously but transcription fluctuates between the two alleles (Levsky and Singer, 2003).

Interchromosomal interactions are also emerging as novel contributors to imprinting decisions. Although imprinting control regions (ICRs) have been characterized as *cis*-regulators of nearby genes, it has recently become clear that they may also act in *trans* (Ling et al., 2006). The ICR on chromosome 7 not only regulates the expression of its flanking Igf2 and H19 loci on the same chromosome but it also interacts with an intergenic region located between the *Wsb1* and *Nf1* genes on chromosome 11. This interaction is mediated by the maternal ICR on chromosome 7 via binding of the boundary element protein CTCF (Ling et al., 2006). At a more global level, the physical interaction of X-chromosome homologs may be important in determining which of the two copies becomes silenced in mammalian X inactivation. Mapping of the location of the two X chromosomes in embryonic stem (ES) cells shows that the two homologs briefly come in close spatial proximity during the period in differentiation when X-inactivation choice occurs (Bacher et al., 2006; Xu et al., 2006). These results clearly point to an emerging role for physical proximity of genome regions in gene regulation.

One of the most important genome functions that is directly affected by the physical organization of the genome is the formation of chromosomal translocations (Meaburn et al., 2006). These occur when unrepaired DSBs from separate chromosomes undergo illegitimate joining. Formation of translocations requires the interaction, and thus physical proximity, of partner chromosomes. Spatial mapping of genome regions that frequently undergo translocations indicates a significant correlation between their proximity and translocation frequency (Bickmore and Teague, 2002; Cornforth et al., 2002; Parada and Misteli, 2002; Figure 3C). The breakage sites of several common translocations, including PML/RAR and BCR/ABL, are more frequently found in close spatial proximity in normal B cells prior to undergoing translocations than would be expected based on random positioning (Lukasova et al., 1997; Neves et al., 1999). A gradual correlation between translocation frequency and spatial proximity is also observed in Burkitt's lymphoma, where the *myc* locus is on average closest to its most frequent translocation partner *IgH*, whereas it is increasingly distal from its two minor translocation partners, *Ig*λ and *Ig*κ (Roix et al., 2003). Furthermore, tissue-specific proximity of chromosomes correlates with tissue-specific translocation frequency (Parada et al., 2004). Additional support for the idea that physical proximity enhances the formation of chromosomal translocations comes from the observation that the degree of intermingling amongst adjacent chromosomes strongly correlates with translocation frequency (Branco and Pombo, 2006).

A similar role for proximity has been implicated in recombination. Repair of DSBs by nonhomologous end joining or homologous recombination occurs significantly more efficiently between sites located on the same chromosome, which by definition are in close spatial proximity, than between loci on separate chromosomes (Richardson and Jasin, 2000; D'Anjou et al., 2004). In *S. cerevisiae* the MATa locus is on average in closer spatial proximity to its preferred recombination partner HML compared to its roughly equally distant, but less favored, partner HMR located on the same chromosome (Bressan et al., 2004). Interestingly, in *S. cerevisiae* there is no difference in the efficiency of intra- and interchromosomal rejoining of DSBs (Haber and Leung, 1996). This fact is most likely due to the fundamentally different nature of chromosome organization whereby mammalian chromosomes are confined to defined subvolumes of the nucleus, which are referred to as chromosome territories, but *S. cerevisiae* chromosomes appear to lack such territoriality (Haber and Leung, 1996).

Models of Cellular Organization of Genome Function

We have accumulated a considerable amount of information about the multiple levels of genome organization and nuclear architecture. But can we derive a comprehensive model of how genomes are organized and function in vivo? Such a model should account for the complex morphological features of the nucleus and should be consistent with the structural and dynamic properties of genomes. Two types of models should be considered: deterministic models and self-organizing models.

Deterministic Models

In a deterministic model, structure dictates function. Architectural features, such as compartments, are purposefully built from dedicated structural elements to provide an environment for a particular process (Figure 4A). Such a compartment is defined by stable structural elements, and its presence is independent of the ongoing function (Figure 4A).

Deterministic models of nuclear function are consistent with the observation of several relatively stable structures within the cell nucleus, such as the lamin network, the presence of short actin filaments, or the nuclear bodies, all of which might serve as structural scaffolds (Gruenbaum et al., 2005; McDonald et al., 2006). However, no dedicated

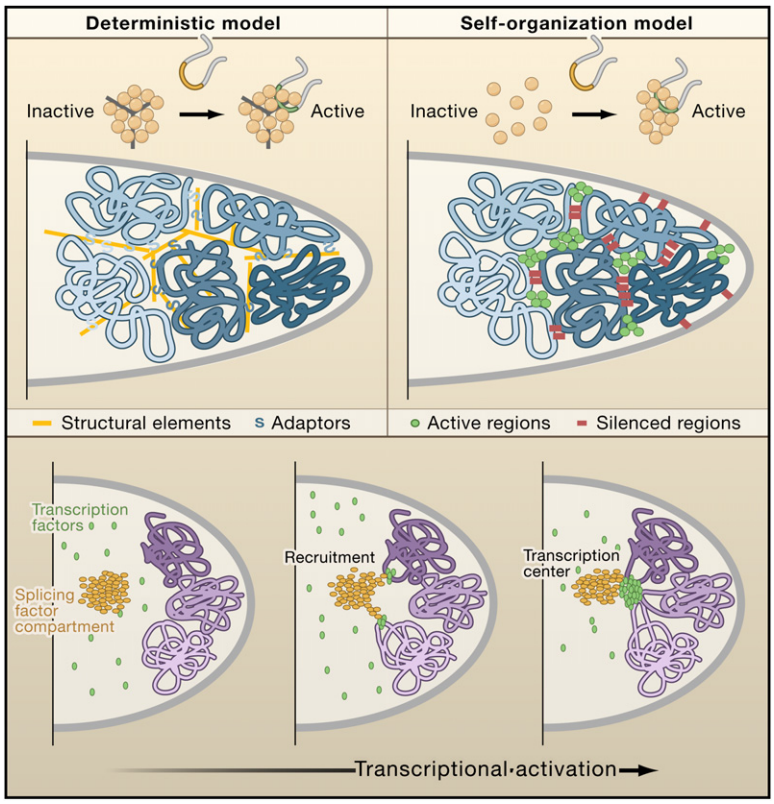

Figure 4. Models of Nuclear Organization

(Top left) In a deterministic model a functional site (transcription, for example) is preformed and contains structural elements. In this model, chromosome position is established and maintained by specific interactions of chromosomes with a scaffold.

(Top right) In a self-organization model the site forms around a poised gene as a consequence of its activation. In this model, chromosome position is determined by the interaction of functionally equivalent regions on distinct chromosomes.

(Bottom) Nuclear architecture is generated by self-organization. Transcription factors are predominantly unbound and diffuse freely though the nucleus in search of specific binding sites. Upon initial transcriptional activation of a particular gene, chromatin is remodeled, and transcription factors are recruited to the gene where they initiate formation of a transcription hub. As pre-mRNA is synthesized, splicing factors are recruited from their storage compartments. At high levels of transcription, multiple genes may coalesce to form a transcription center, which is closely associated with the splicing-factor compartment. The formation of the transcription center does not require the presence of a nuclear scaffold; chromatin is sufficient to serve as an attachment site. The configuration of splicing-factor compartment, transcription factory, and gene locus is generated in a self-organizing manner without the requirement for dedicated structural elements.

structural elements have been identified for any of the nuclear compartments, and the functional role of nuclear scaffolds is unclear. Elimination of some of the prime structural components of the nucleus, such as the lamins, has relatively little effect on the spatial organization of transcription and pre-mRNA splicing sites, although interference with the essential B-type lamins affects transcription and splicing (Spann et al., 1997; Sullivan et al., 1999; Vecerova et al., 2004). Along the same lines, although interference with nuclear actin-filament formation reduces transcription levels, the effect is moderate, and no global reorganization of transcription sites occurs (McDonald et al., 2006).

The same concerns apply to deterministic mechanisms of spatial genome organization (Figure 4, top). It is not trivial to think of mechanisms by which chromosomes are positioned in a specified, nonrandom manner. Such mechanisms would require recognition of each chromosome individually and their arrangement in particular patterns (Figure 4, top). No such recognition mechanisms are known. In fact, the observation that the chromosome-positioning patterns are not well conserved between cells in a population but are largely probabilistic suggests that no such mechanisms exist (Parada and Misteli, 2002; Cremer et al., 2006). Furthermore, the fact that chromosome-positioning patterns differ amongst cell types and tissue types would imply the existence of cell-type-specific organizing mechanisms; this seems unlikely.

A prediction of deterministic models is that structural elements should form prior to commencement of activities within those structures. The reassembly of the nucleolus after mitosis is a good example to test this prediction. Ribosomal gene expression ceases during mitosis and resumes at the telophase/G1 boundary. Rather than first forming a nucleolus into which ribosomal genes are recruited, the reforming nucleolus is nucleated around the reactivated ribosomal genes and then increases gradually in size concomitant with resumption of rRNA transcription, which strongly suggests that the structure of the nucleolus is interdependent on its function (Hernandez-Verdun et al., 2002). In sum, although the complex architecture of the genome and of nuclear processes seems to make a compelling case for deterministic organization, much of the current experimental evidence does not support such a model.

Self-Organization Models

Many nuclear properties, particularly recently discovered ones, are compatible with self-organization, and it has been suggested that the nucleus as a whole is a self-organizing system (Misteli, 2001a; Cook, 2002). Such systems are based on the dynamic interaction of their components and the mutual interplay between structure and

function. The morphological appearance and spatial organization of a self-organizing system is a reflection of the sum of all ongoing functions. At the same time the resulting structural features support and enhance ongoing activities in a self-reinforcing manner (Figure 4, top).

Evidence for self-organization of nuclear architecture and function exists at all levels of organization. Interference with virtually any nuclear process, including transcription, pre-mRNA splicing, and replication, leads to rapid changes in global architecture (Lamond and Spector, 2003). Furthermore, when new functional sites are generated within the nuclear space, structural elements often form de novo. A classic example is the ectopic expression of ribosomal genes on plasmids, which leads to the biogenesis of micronucleoli (Karpen et al., 1988). Similarly, replication factories form rapidly from dynamic components at replication origins, repair foci form upon induction of DSBs, and activation of genes may initiate the formation of transcription hubs (Houtsmuller et al., 1999; Sporbert et al., 2002; Chakalova et al., 2005). Therefore, several of the most prominent nuclear structures can form de novo, which is a hallmark of self-organizing systems.

The spatial positioning of genes and chromosomes can similarly be explained by self-organizing properties. The central idea is that the sum of all functional properties of a chromosome (i.e., the frequency and linear distribution of its active and inactive regions) determines its positioning. It can be envisioned that functionally equivalent regions from multiple chromosomes cluster within the nucleus. It is well-known that heterochromatic regions on distinct chromosomes frequently cluster in 3D space. Similarly, active chromosome regions may be constrained by shared transcription factories (Cook, 2002; Chakalova et al., 2005). Quantitative analysis demonstrates that the organization of chromatin fibers into loops constrained by transcription and replication factories represents a favorable arrangement and creates an entropy minimum, thus stabilizing the system overall (Marenduzzo et al., 2006). The sum of these interactions creates preferential associations amongst genome regions and chromosomes and constrains their motion. In this way, each genome region and each chromosome determines in a self-organizing fashion whom its neighbor is, and preferential, yet probabilistic, patterns of positioning emerge.

It is important to realize that self-organization models of nuclear architecture are not contradictory to the presence of relatively stable structures such as a lamina or a putative actin-based nucleoskeleton (Gruenbaum et al., 2005; McDonald et al., 2006). Stable structures may still serve as platforms onto which functional sites are assembled. Although such scaffolds may enhance the efficiency of nuclear processes, they might not be required; rather, the structural integrity of the nucleus might largely be generated by chromatin itself. Although it is generally assumed that transcription and replication factories are tethered to a nucleoskeleton of unknown identity, it is equally possible that chromatin itself serves as the attachment site. It is, for example, plausible that a transcription factory forms de novo on chromatin upon initiation of transcription and then attracts other transcribed genes to form a multigene transcription hub (Cook, 2002; Chakalova et al., 2005; Figure 4, bottom). Consistent with such a chromatin-driven self-organization scenario, it is well accepted that replication and DNA-repair machineries use chromatin as their nucleation site rather than as a dedicated nuclear scaffold.

The obvious weakness of self-organization models is the difficulty of testing them experimentally. Although in deterministically organized systems, structure and function can be separated and molecularly characterized, the intimate structure-function interplay in self-organizing systems prevents uncoupling by experimental means. Although much of the experimental data are consistent with self-organization, other approaches must be used to probe the self-organizing nature of genome organization and nuclear architecture. A promising strategy is the use of computational models. Sufficient data are being accumulated to constrain computational models and to make testable quantitative predictions (Gorski and Misteli, 2005). The first simple applications of these strategies are now being developed, and initial results indicate that the morphological appearance of nuclear-splicing-factor compartments can indeed be modeled by assuming principles of self-organization (Soula et al., 2005; Carrero et al., 2006).

Conclusions

The deceivingly simple question of how genomes function has become the Holy Grail of modern biology. Although sequencing efforts, molecular analysis, and in vitro biochemistry have identified the key players in virtually all genome processes, we have come to appreciate the importance of cellular organization in genome function. The degree of structural complexity in the mammalian cell nucleus is stunning. At first glance, the nonrandom organization of genomes and their interacting factors appears to complicate the task of coordinating genome functions as processes are compartmentalized and the appropriate components must be present in just the right place and at the right time to ensure efficient gene function. On the other hand, these apparent complications are counterbalanced by their potential as regulatory mechanisms. It is now clear that process compartmentalization, chromatin accessibility, and spatial sequestration of genes and their regulatory factors serve to modulate the output and functional status of genomes. New system-wide models of how genomes function in vivo based on stochastic and self-organizing behavior are emerging, and they must now be tested by comparing complete maps of transcriptional activity, epigenetic modifications, chromatin structure, and spatial positioning with cellular genome organization. The complex nature of these models requires a novel theoretical framework of biological processes and new experimental approaches, including visualization technology, analysis of dynamic events, and system-wide computational modeling, to test them. The

exploration of the principles of cellular genome organization and function will be one of the great challenges of this new kind of cell biology.

ACKNOWLEDGMENTS

I thank members of the Misteli and Hager laboratories for critical comments and R. Chiffer for editorial assistance with the manuscript. Work in the author's laboratory is supported by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research.

REFERENCES

Agresti, A., Scaffidi, P., Riva, A., Caiolfa, V.R., and Bianchi, M.E. (2005). GR and HMGB1 interact only within chromatin and influence each other's residence time. Mol. Cell *18*, 109–121.

Ansari, A., and Hampsey, M. (2005). A role for the CPF 3′-end processing machinery in RNAP II-dependent gene looping. Genes Dev. *19*, 2969–2978.

Bacher, C.P., Guggiari, M., Brors, B., Augui, S., Clerc, P., Avner, P., Eils, R., and Heard, E. (2006). Transient colocalization of X-inactivation centres accompanies the initiation of X inactivation. Nat. Cell Biol. *8*, 293–299.

Becker, M., Baumann, C., John, S., Walker, D.A., Vigneron, M., McNally, J.G., and Hager, G.L. (2002). Dynamic behavior of transcription factors on a natural promoter in living cells. EMBO Rep. *3*, 1188–1194.

Bekker-Jensen, S., Lukas, C., Kitagawa, R., Melander, F., Kastan, M.B., Bartek, J., and Lukas, J. (2006). Spatial organization of the mammalian genome surveillance machinery in response to DNA strand breaks. J. Cell Biol. *173*, 195–206.

Bentley, D.L. (2005). Rules of engagement: co-transcriptional recruitment of pre-mRNA processing factors. Curr. Opin. Cell Biol. *17*, 251–256.

Bickmore, W.A., and Teague, P. (2002). Influences of chromosome size, gene density and nuclear position on the frequency of constitutional translocations in the human population. Chromosome Res. *10*, 707–715.

Blanton, J., Gaszner, M., and Schedl, P. (2003). Protein:protein interactions and the pairing of boundary elements in vivo. Genes Dev. *17*, 664–675.

Bolzer, A., Kreth, G., Solovei, I., Koehler, D., Saracoglu, K., Fauth, C., Muller, S., Eils, R., Cremer, C., Speicher, M.R., and Cremer, T. (2005). Three-dimensional maps of all chromosomes in human male fibroblast nuclei and prometaphase rosettes. PLoS Biol. *3*, e157. 10.1371/journal.pbio.0030157.

Bosisio, D., Marazzi, I., Agresti, A., Shimizu, N., Bianchi, M.E., and Natoli, G. (2006). A hyper-dynamic equilibrium between promoter-bound and nucleoplasmic dimers controls NF-kappaB-dependent gene activity. EMBO J. *25*, 798–810.

Boutanaev, A.M., Mikhaylova, L.M., and Nurminsky, D.I. (2005). The pattern of chromosome folding in interphase is outlined by the linear gene density profile. Mol. Cell. Biol. *25*, 8379–8386.

Boyle, S., Gilchrist, S., Bridger, J.M., Mahy, N.L., Ellis, J.A., and Bickmore, W.A. (2001). The spatial organization of human chromosomes within the nuclei of normal and emerin-mutant cells. Hum. Mol. Genet. *10*, 211–219.

Branco, M.R., and Pombo, A. (2006). Intermingling of chromosome territories in interphase suggests role in translocations and transcription-dependent associations. PLoS Biol. *4*, e138. 10.1371/journal.pbio.0040138.

Bressan, D.A., Vazquez, J., and Haber, J.E. (2004). Mating type-dependent constraints on the mobility of the left arm of yeast chromosome III. J. Cell Biol. *164*, 361–371.

Brickner, J.H., and Walter, P. (2004). Gene recruitment of the activated INO1 locus to the nuclear membrane. PLoS Biol. *2*, e342. 10.1371/journal.pbio.0020342.

Brown, J.M., Leach, J., Reittie, J.E., Atzberger, A., Lee-Prudhoe, J., Wood, W.G., Higgs, D.R., Iborra, F.J., and Buckle, V.J. (2006). Coregulated human globin genes are frequently in spatial proximity when active. J. Cell Biol. *172*, 177–187.

Byrd, K., and Corces, V.G. (2003). Visualization of chromatin domains created by the gypsy insulator of Drosophila. J. Cell Biol. *162*, 565–574.

Cabal, G.G., Genovesio, A., Rodriguez-Navarro, S., Zimmer, C., Gadal, O., Lesne, A., Buc, H., Feuerbach-Fournier, F., Olivo-Marin, J.C., Hurt, E.C., and Nehrbass, U. (2006). SAGA interacting factors confine sub-diffusion of transcribed genes to the nuclear envelope. Nature *441*, 770–773.

Cai, S., Han, H.J., and Kohwi-Shigematsu, T. (2003). Tissue-specific nuclear architecture and gene expression regulated by SATB1. Nat. Genet. *34*, 42–51.

Cai, S., Lee, C.C., and Kohwi-Shigematsu, T. (2006). SATB1 packages densely looped, transcriptionally active chromatin for coordinated expression of cytokine genes. Nat. Genet. *38*, 1278–1288.

Carrero, G., Hendzel, M.J., and de Vries, G. (2006). Modelling the compartmentalization of splicing factors. J. Theor. Biol. *239*, 298–312.

Casolari, J.M., Brown, C.R., Komili, S., West, J., Hieronymus, H., and Silver, P.A. (2004). Genome-wide localization of the nuclear transport machinery couples transcriptional status and nuclear organization. Cell *117*, 427–439.

Chakalova, L., Debrand, E., Mitchell, J.A., Osborne, C.S., and Fraser, P. (2005). Replication and transcription: shaping the landscape of the genome. Nat. Rev. Genet. *6*, 669–677.

Chambeyron, S., and Bickmore, W.A. (2004). Chromatin decondensation and nuclear reorganization of the HoxB locus upon induction of transcription. Genes Dev. *18*, 1119–1130.

Cheutin, T., McNairn, A.J., Jenuwein, T., Gilbert, D.M., Singh, P.B., and Misteli, T. (2003). Maintenance of stable heterochromatin domains by dynamic HP1 binding. Science *299*, 721–725.

Cheutin, T., Gorski, S.A., May, K.M., Singh, P.B., and Misteli, T. (2004). In vivo dynamics of Swi6 in yeast: Evidence for a stochastic model of heterochromatin. Mol. Cell. Biol. *24*, 3157–3167.

Chubb, J.R., and Bickmore, W.A. (2003). Considering nuclear compartmentalization in the light of nuclear dynamics. Cell *112*, 403–406.

Cook, P.R. (1999). Organization of replication and transcription. Science *284*, 1790–1795.

Cook, P.R. (2002). Predicting three-dimensional genome structure from transcriptional activity. Nat. Genet. *32*, 347–352.

Cornforth, M.N., Greulich-Bode, K.M., Loucas, B.D., Arsuaga, J., Vazquez, M., Sachs, R.K., Bruckner, M., Molls, M., Hahnfeldt, P., Hlatky, L., and Brenner, D.J. (2002). Chromosomes are predominantly located randomly with respect to each other in interphase human cells. J. Cell Biol. *159*, 237–244.

Cremer, M., Kupper, K., Wagler, B., Wizelman, L., Hase Jv, J., Weiland, Y., Kreja, L., Diebold, J., Speicher, M.R., and Cremer, T. (2003). Inheritance of gene density-related higher order chromatin arrangements in normal and tumor cell nuclei. J. Cell Biol. *162*, 809–820.

Cremer, T., Cremer, M., Dietzel, S., Muller, S., Solovei, I., and Fakan, S. (2006). Chromosome territories - a functional nuclear landscape. Curr. Opin. Cell Biol. *18*, 307–316.

D'Anjou, H., Chabot, C., and Chartrand, P. (2004). Preferential accessibility to specific genomic loci for the repair of double-strand breaks in human cells. Nucleic Acids Res. *32*, 6136–6143.

Dillon, N., and Festenstein, R. (2002). Unravelling heterochromatin: competition between positive and negative factors regulates accessibility. Trends Genet. *18*, 252–258.

Dundr, M., Hoffmann-Rohrer, U., Hu, Q., Grummt, I., Rothblum, L.I., Phair, R.D., and Misteli, T. (2002). A kinetic framework for a mammalian RNA polymerase in vivo. Science *298*, 1623–1626.

Elbi, C., Misteli, T., and Hager, G.L. (2002). Recruitment of dioxin receptor to active transcription sites. Mol. Biol. Cell *13*, 2001–2015.

Essers, J., Vermeulen, W., and Houtsmuller, A.B. (2006). DNA damage repair: anytime, anywhere? Curr. Opin. Cell Biol. *18*, 240–246.

Ferreira, J., Paolella, G., Ramos, C., and Lamond, A.I. (1997). Spatial organization of large-scale chromatin domains in the nucleus: A magnified view of single chromosome territories. J. Cell Biol. *139*, 1597–1610.

Festenstein, R., Pagakis, S.N., Hiragami, K., Lyon, D., Verreault, A., Sekkali, B., and Kioussis, D. (2003). Modulation of heterochromatin protein 1 dynamics in primary Mammalian cells. Science *299*, 719–721.

Fraser, P. (2006). Transcriptional control thrown for a loop. Curr. Opin. Genet. Dev. *16*, 490–495.

Gartenberg, M.R., Neumann, F.R., Laroche, T., Blaszczyk, M., and Gasser, S.M. (2004). Sir-mediated repression can occur independently of chromosomal and subnuclear contexts. Cell *119*, 955–967.

Gilbert, N., Boyle, S., Fiegler, H., Woodfine, K., Carter, N.P., and Bickmore, W.A. (2004). Chromatin architecture of the human genome: gene-rich domains are enriched in open chromatin fibers. Cell *118*, 555–566.

Gorisch, S.M., Wachsmuth, M., Toth, K.F., Lichter, P., and Rippe, K. (2005). Histone acetylation increases chromatin accessibility. J. Cell Sci. *118*, 5825–5834.

Gorski, S., and Misteli, T. (2005). Systems biology in the cell nucleus. J. Cell Sci. *118*, 4083–4092.

Gruenbaum, Y., Margalit, A., Goldman, R.D., Shumaker, D.K., and Wilson, K.L. (2005). The nuclear lamina comes of age. Nat. Rev. Mol. Cell Biol. *6*, 21–31.

Haber, J.E., and Leung, W.Y. (1996). Lack of chromosome territoriality in yeast: promiscuous rejoining of broken chromosome ends. Proc. Natl. Acad. Sci. USA *93*, 13949–13954.

Hager, G.L., Elbi, C., and Becker, M. (2002). Protein dynamics in the nuclear compartment. Curr. Opin. Genet. Dev. *12*, 137–141.

Hancock, R. (2004). A role for macromolecular crowding effects in the assembly and function of compartments in the nucleus. J. Struct. Biol. *146*, 281–290.

Hernandez-Verdun, D., Roussel, P., and Gebrane-Younes, J. (2002). Emerging concepts of nucleolar assembly. J. Cell Sci. *115*, 2265–2270.

Horike, S., Cai, S., Miyano, M., Cheng, J.F., and Kohwi-Shigematsu, T. (2005). Loss of silent-chromatin looping and impaired imprinting of DLX5 in Rett syndrome. Nat. Genet. *37*, 31–40.

Houtsmuller, A.B., Rademakers, S., Nigg, A.L., Hoogstraten, D., Hoeijmakers, J.H., and Vermeulen, W. (1999). Action of DNA repair endonuclease ERCC1/XPF in living cells. Science *284*, 958–961.

Ishii, K., Arib, G., Lin, C., Van Houwe, G., and Laemmli, U.K. (2002). Chromatin boundaries in budding yeast: the nuclear pore connection. Cell *109*, 551–562.

Karpen, G.H., Schaefer, J.E., and Laird, C.D. (1988). A Drosophila rRNA gene located in euchromatin is active in transcription and nucleolus formation. Genes Dev. *2*, 1745–1763.

Kim, S.H., McQueen, P.G., Lichtman, M.K., Shevach, E.M., Parada, L.A., and Misteli, T. (2004). Spatial genome organization during T-cell differentiation. Cytogenet. Genome Res. *105*, 292–301.

Kitamura, E., Blow, J.J., and Tanaka, T.U. (2006). Live-cell imaging reveals replication of individual replicons in eukaryotic replication factories. Cell *125*, 1297–1308.

Kosak, S.T., Skok, J.A., Medina, K.L., Riblet, R., Le Beau, M.M., Fisher, A.G., and Singh, H. (2002). Subnuclear compartmentalization of immunoglobulin loci during lymphocyte development. Science *296*, 158–162.

Labrador, M., and Corces, V.G. (2002). Setting the boundaries of chromatin domains and nuclear organization. Cell *111*, 151–154.

Lamond, A.I., and Spector, D.L. (2003). Nuclear speckles: a model for nuclear organelles. Nat. Rev. Mol. Cell Biol. *4*, 605–612.

Levsky, J.M., and Singer, R.H. (2003). Gene expression and the myth of the average cell. Trends Cell Biol. *13*, 4–6.

Ling, J.Q., Li, T., Hu, J.F., Vu, T.H., Chen, H.L., Qiu, X.W., Cherry, A.M., and Hoffman, A.R. (2006). CTCF mediates interchromosomal colocalization between Igf2/H19 and Wsb1/Nf1. Science *312*, 269–272.

Lisby, M., Mortensen, U.H., and Rothstein, R. (2003). Colocalization of multiple DNA double-strand breaks at a single Rad52 repair centre. Nat. Cell Biol. *5*, 572–577.

Lomvardas, S., Barnea, G., Pisapia, D.J., Mendelsohn, M., Kirkland, J., and Axel, R. (2006). Interchromosomal interactions and olfactory receptor choice. Cell *126*, 403–413.

Lukasova, E., Kozubek, S., Kozubek, M., Kjeronska, J., Ryznar, L., Horakova, J., Krahulcova, E., and Horneck, G. (1997). Localisation and distance between ABL and BCR genes in interphase nuclei of bone marrow cells of control donors and patients with chronic myeloid leukaemia. Hum. Genet. *100*, 525–535.

Marenduzzo, D., Micheletti, C., and Cook, P.R. (2006). Entropy-driven genome organization. Biophys. J. *90*, 3712–3721.

Martin, M., Cho, J., Cesare, A.J., Griffith, J.D., and Attardi, G. (2005). Termination factor-mediated DNA loop between termination and initiation sites drives mitochondrial rRNA synthesis. Cell *123*, 1227–1240.

McDonald, D., Carrero, G., Andrin, C., de Vries, G., and Hendzel, M.J. (2006). Nucleoplasmic beta-actin exists in a dynamic equilibrium between low-mobility polymeric species and rapidly diffusing populations. J. Cell Biol. *172*, 541–552.

McNairn, A.J., Okuno, Y., Misteli, T., and Gilbert, D.M. (2005). Chinese hamster ORC subunits dynamically associate with chromatin throughout the cell-cycle. Exp. Cell Res. *308*, 345–356.

McNally, J.G., Muller, W.G., Walker, D., Wolford, R., and Hager, G.L. (2000). The glucocorticoid receptor: Rapid exchange with regulatory sites in living cells. Science *287*, 1262–1265.

Meaburn, K.J., and Misteli, T. (2007). Chromosome territories. Nature *445*, 379–381.

Meaburn, K.J., Misteli, T., and Soutoglou, E. (2006). Spatial genome organization in the formation of chromosomal translocations. Semin. Cancer Biol.. Published online October 26, 2006. 10.1016/j.semcancer.2006.10.008.

Minton, A.P. (2000). Implications of macromolecular crowding for protein assembly. Curr. Opin. Struct. Biol. *10*, 34–39.

Misteli, T. (2001a). The concept of self-organization in cellular architecture. J. Cell Biol. *155*, 181–185.

Misteli, T. (2001b). Protein dynamics: Implications for nuclear architecture and gene expression. Science *291*, 843–847.

Misteli, T. (2005). Concepts in nuclear architecture. Bioessays *27*, 477–487.

Murrell, A., Heeson, S., and Reik, W. (2004). Interaction between differentially methylated regions partitions the imprinted genes Igf2 and H19 into parent-specific chromatin loops. Nat. Genet. *36*, 889–893.

Neves, H., Ramos, C., da Silva, M.G., Parreira, A., and Parreira, L. (1999). The nuclear topography of ABL, BCR, PML, and RARalpha

genes: evidence for gene proximity in specific phases of the cell cycle and stages of hematopoietic differentiation. Blood *93*, 1197–1207.

Noma, K., Cam, H.P., Maraia, R.J., and Grewal, S.I. (2006). A role for TFIIIC transcription factor complex in genome organization. Cell *125*, 859–872.

Osborne, C.S., Chakalova, L., Brown, K.E., Carter, D., Horton, A., Debrand, E., Goyenechea, B., Mitchell, J.A., Lopes, S., Reik, W., and Fraser, P. (2004). Active genes dynamcially colocalize to shared sites of ongoing transcription. Nat. Genet. *36*, 1065–1071.

O'Sullivan, J.M., Tan-Wong, S.M., Morillon, A., Lee, B., Coles, J., Mellor, J., and Proudfoot, N.J. (2004). Gene loops juxtapose promoters and terminators in yeast. Nat. Genet. *36*, 1014–1018.

Palstra, R.J., Tolhuis, B., Splinter, E., Nijmeijer, R., Grosveld, F., and de Laat, W. (2003). The beta-globin nuclear compartment in development and erythroid differentiation. Nat. Genet. *35*, 190–194.

Parada, L., and Misteli, T. (2002). Chromosome positioning in the interphase nucleus. Trends Cell Biol. *12*, 425–432.

Parada, L., McQueen, P., and Misteli, T. (2004). Tissue-specific spatial organization of genomes. Genome Biol. *7*, R44.

Phair, R.D., Scaffidi, P., Elbi, C., Vecerova, J., Dey, A., Ozato, K., Brown, D.T., Hager, G., Bustin, M., and Misteli, T. (2004). Global nature of dynamic protein-chromatin interactions in vivo: three-dimensional genome scanning and dynamic interaction networks of chromatin proteins. Mol. Cell. Biol. *24*, 6393–6402.

Pickersgill, H., Kalverda, B., de Wit, E., Talhout, W., Fornerod, M., and van Steensel, B. (2006). Characterization of the Drosophila melanogaster genome at the nuclear lamina. Nat. Genet. *38*, 1005–1014.

Politi, A., Mone, M.J., Houtsmuller, A.B., Hoogstraten, D., Vermeulen, W., Heinrich, R., and van Driel, R. (2005). Mathematical modeling of nucleotide excision repair reveals efficiency of sequential assembly strategies. Mol. Cell *19*, 679–690.

Ragoczy, T., Bender, M.A., Telling, A., Byron, R., and Groudine, M. (2006). The locus control region is required for association of the murine beta-globin locus with engaged transcription factories during erythroid maturation. Genes Dev. *20*, 1447–1457.

Raska, I., Shaw, P.J., and Cmarko, D. (2006). Structure and function of the nucleolus in the spotlight. Curr. Opin. Cell Biol. *18*, 325–334.

Richardson, C., and Jasin, M. (2000). Frequent chromosomal translocations induced by DNA double-strand breaks. Nature *405*, 697–700.

Roix, J.J., McQueen, P.G., Munson, P.J., Parada, L.A., and Misteli, T. (2003). Spatial proximity of translocation-prone gene loci in human lymphomas. Nat. Genet. *34*, 287–291.

Sabo, P.J., Humbert, R., Hawrylycz, M., Wallace, J.C., Dorschner, M.O., McArthur, M., and Stamatoyannopoulos, J.A. (2004). Genome-wide identification of DNaseI hypersensitive sites using active chromatin sequence libraries. Proc. Natl. Acad. Sci. USA *101*, 4537–4542.

Sadoni, N., Langer, S., Fauth, C., Bernardi, G., Cremer, T., Turner, B.M., and Zink, D. (1999). Nuclear organization of mammalian genomes. Polar chromosome territories build up functionally distinct higher order compartments. J. Cell Biol. *146*, 1211–1226.

Scaffidi, P., and Misteli, T. (2005). Reversal of the cellular phenotype in the premature aging disease Hutchinson-Gilford progeria syndrome. Nat. Med. *11*, 440–445.

Schneider, D.A., and Nomura, M. (2004). RNA polymerase I remains intact without subunit exchange through multiple rounds of transcription in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA *101*, 15112–15117.

Shopland, L.S., Lynch, C.R., Peterson, K.A., Thornton, K., Kepper, N., Hase, J., Stein, S., Vincent, S., Molloy, K.R., Kreth, G., et al. (2006). Folding and organization of a contiguous chromosome region according to the gene distribution pattern in primary genomic sequence. J. Cell Biol. *174*, 27–38.

Shumaker, D.K., Dechat, T., Kohlmaier, A., Adam, S.A., Bozovsky, M.R., Erdos, M.R., Eriksson, M., Goldman, A.E., Khuon, S., Collins, F.S., et al. (2006). Mutant nuclear lamin A leads to progressive alterations of epigenetic control in premature aging. Proc. Natl. Acad. Sci. USA *103*, 8703–8708.

Simonis, M., Klous, P., Splinter, E., Moshkin, Y., Willemsen, R., de Wit, E., van Steensel, B., and de Laat, W. (2006). Nuclear organization of active and inactive chromatin domains uncovered by chromosome conformation capture-on-chip (4C). Nat. Genet. *38*, 1348–1354.

Soula, H., Robardet, C., Perrin, F., Gripon, S., Beslon, G., and Gandrillon, O. (2005). Modeling the emergence of multi-protein dynamic structures by principles of self-organization through the use of 3DSpi, a multi-agent-based software. BMC Bioinformatics *6*, 228.

Spann, T.P., Moir, R.D., Goldman, A.E., Stick, R., and Goldman, R.D. (1997). Disruption of nuclear lamin organization alters the distribution of replication factors and inhibits DNA synthesis. J. Cell Biol. *136*, 1201–1212.

Spilianakis, C.G., Lalioti, M.D., Town, T., Lee, G.R., and Flavell, R.A. (2005). Interchromosomal associations between alternatively expressed loci. Nature *435*, 637–645.

Sporbert, A., Gahl, A., Ankerhold, R., Leonhardt, H., and Cardoso, M.C. (2002). DNA polymerase clamp shows little turnover at established replication sites but sequential de novo assembly at adjacent origin clusters. Mol. Cell *10*, 1355–1365.

Stenoien, D.L., Patel, K., Mancini, M.G., Dutertre, M., Smith, C.L., O'Malley, B.W., and Mancini, M.A. (2001). FRAP reveals that mobility of oestrogen receptor-a is ligand and proteasome-dependent. Nat. Cell Biol. *3*, 15–23.

Sullivan, T., Escalante-Alcalde, D., Bhatt, H., Anver, M., Bhat, N., Nagashima, K., Stewart, C.L., and Burke, B. (1999). Loss of A-type lamin expression compromises nuclear envelope integrity leading to muscular dystrophy. J. Cell Biol. *147*, 913–920.

Taddei, A., Van Houwe, G., Hediger, F., Kalck, V., Cubizolles, F., Schober, H., and Gasser, S.M. (2006). Nuclear pore association confers optimal expression levels for an inducible yeast gene. Nature *441*, 774–778.

Therizols, P., Fairhead, C., Cabal, G.G., Genovesio, A., Olivo-Marin, J.C., Dujon, B., and Fabre, E. (2006). Telomere tethering at the nuclear periphery is essential for efficient DNA double strand break repair in subtelomeric region. J. Cell Biol. *172*, 189–199.

Thompson, M., Haeusler, R.A., Good, P.D., and Engelke, D.R. (2003). Nucleolar clustering of dispersed tRNA genes. Science *302*, 1399–1401.

van Driel, R., Fransz, P.F., and Verschure, P.J. (2003). The eukaryotic genome: a system regulated at different hierarchical levels. J. Cell Sci. *116*, 4067–4075.

Vecerova, J., Koberna, K., Malinsky, J., Soutoglou, E., Sullivan, T., Stewart, C.L., Raska, I., and Misteli, T. (2004). Formation of nuclear splicing factor compartments is independent of lamins A/C. Mol. Biol. Cell. *15*, 4904–4910.

Verschure, P.J., Van Der Kraan, I., Manders, E.M., Hoogstraten, D., Houtsmuller, A.B., and Van Driel, R. (2003). Condensed chromatin domains in the mammalian nucleus are accessible to large macromolecules. EMBO Rep. *4*, 861–866.

Volpi, E.V., Chevret, E., Jones, T., Vatcheva, R., Williamson, J., Beck, S., Campbell, R.D., Goldsworthy, M., Powis, S.H., Ragoussis, J., et al. (2000). Large-scale chromatin organization of the major histocompatibility complex and other regions of human chromosome 6 and its response to interferon in interphase nuclei. J. Cell Sci. *113*, 1565–1576.

Wansink, D.G., Schul, W., van der Kraan, I., van Steensel, B., van Driel, R., and de Jong, L. (1993). Fluorescent labelling of nascent RNA reveals transcription by RNA polymerase II in domains scattered throughout the nucleus. J. Cell Biol. *122*, 282–293.

Weil, M.R., Widlak, P., Minna, J.D., and Garner, H.R. (2004). Global survey of chromatin accessibility using DNA microarrays. Genome Res. *14*, 1374–1381.

Wijgerde, M., Grosveld, F., and Fraser, P. (1995). Transcription complex stability and chromatin dynamics in vivo. Nature *377*, 209–213.

Williams, R.R., Broad, S., Sheer, D., and Ragoussis, J. (2002). Subchromosomal positioning of the epidermal differentiation complex (EDC) in keratinocyte and lymphoblast interphase nuclei. Exp. Cell Res. *272*, 163–175.

Woodcock, C.L. (2006). Chromatin architecture. Curr. Opin. Struct. Biol. *16*, 213–220.

Wurtele, H., and Chartrand, P. (2006). Genome-wide scanning of HoxB1-associated loci in mouse ES cells using an open-ended Chromosome Conformation Capture methodology. Chromosome Res. *14*, 477–495.

Xu, N., Tsai, C.L., and Lee, J.T. (2006). Transient homologous chromosome pairing marks the onset of X inactivation. Science *311*, 1149–1152.

Yao, J., Munson, K.M., Webb, W.W., and Lis, J.T. (2006). Dynamics of heat shock factor association with native gene loci in living cells. Nature *442*, 1050–1053.

Yusufzai, T.M., Tagami, H., Nakatani, Y., and Felsenfeld, G. (2004). CTCF tethers an insulator to subnuclear sites, suggesting shared insulator mechanisms across species. Mol. Cell *13*, 291–298.

Zhao, Z., Tavoosidana, G., Sjolinder, M., Gondor, A., Mariano, P., Wang, S., Kanduri, C., Lezcano, M., Sandhu, K.S., Singh, U., et al. (2006). Circular chromosome conformation capture (4C) uncovers extensive networks of epigenetically regulated intra- and interchromosomal interactions. Nat. Genet. *38*, 1341–1347.

Zink, D., Amaral, M.D., Englmann, A., Land, S., Clarke, L.A., Rudolph, C., Alt, F., Luther, K., Braz, C., Sadoni, N., et al. (2004). Transcription-dependent spatial arrangement of CFTR and adjacent genes in human cell nuclei. J. Cell Biol. *I166*, 815–825.